

BIOCHEMICAL CHARACTERIZATION OF SMALL MOLECULES TARGETING RAL GTPASE

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The Ral subfamily of GTPases consists of highly similar RalA and RalB isoforms that participate in diverse cellular functions including endocytosis, exocytosis, actin cytoskeletal dynamics, and transcription. A large body of evidence has implicated Ral GTPases with tumor cell growth, migration, and angiogenesis in bladder, prostate, lung, and pancreatic cancer. The purpose of this project was to target the activity of Ral GTPases and their association with effector proteins through the identification of small molecule inhibitors that block this interaction. In order to accomplish this, both direct binding to RalB as well as disruption of protein-protein interaction were investigated. The top 200 compounds from a larger computational library of 500,000 compounds targeting the RalBP1 binding site on RalB were tested. Differential scanning fluorimetry (DSF) was used to measure the degree of direct binding between compound and protein through thermal melting shift. To measure disruption between RalB and RalBP1 by small molecules, a novel enzyme-linked immunosorbent assay (ELISA) was developed. Identification of a few key compounds binding to RalB as well as optimization of an ELISA assay for RalBP1 was accomplished. Further direction of this project would be to utilize the ELISA assay to test inhibition of the protein-protein interaction between RalB and RalBP1 using the top compounds from the DSF trials.

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